**IBACON**
Institut für
Biologische Analytik und
Consulting IBACON GmbH

## Final Report

# Ready Biodegradability of Wacker BS 1701 in a Manometric Respirometry Test

(GLP compliant study based on the Directive 92/69/EEC, C.4-D, 1992 and the OECD No. 301 F, 1992)

**Author: Dr. Johannes Hertl**

**Study Completion Date: January 22, 2001**

**Sponsor**

Wacker-Chemie GmbH
Werk Burghausen
Johannes-Hess-Straße 24
84489 Burghausen
Germany

**Test Facility**

Institut für Biologische Analytik
und Consulting IBACON GmbH
Arheilger Weg 17
64380 Rossdorf
Germany

**Project 9545160**

HESSISCHES MINISTERIUM
FÜR UMWELT, LANDWIRTSCHAFT
UND FORSTEN

# GLP-Bescheinigung

**Bescheinigung**

Hiermit wird bestätigt, daß die Prüfeinrichtung
Institut für Biologische Analytik
in 64380 Roßdorf
Industriestraße 1
(Ort, Anschrift)
der IBACON
(Firma)
am 08. und 09 Dezember 1998
(Datum)

von der für die Überwachung zuständigen Behörden über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden:

Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen
Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen
Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung
Prüfungen zur Bestimmung von Rückständen

**Certificate**

It is hereby certified that the test facility
Insitut für Biologische Analytik
in 64380 Roßdorf
Industriestraße 1
(location, address)
of IBACON
(company name)
on 08. und 09. Dezember 1998
(date)

was inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice:

Physical and chemical properties
and determination of content
Environmental toxicity studies
on aquatic and terrestrial organisms
Behaviour in water soil and air,
Bioaccumulation and metabolism
Residues

Im Auftrag

HESSISCHES MINISTERIUM FÜR UMWELT, LANDWIRTSCHAFT UND FORSTEN

Dr. Hecker

(Dr. Hecker) Wiesbaden, den 20. August 1999

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## 1. Summary


**Title:** Ready Biodegradability of Wacker BS 1701 in a Manometric Respirometry Test

**Guidelines/Recommendations:**
- Commission Directive 92/69/EEC, Method C.4-D of July 31, 1992: Manometric Respirometry Test (EEC Publication No. L 383 A, December 1992)
- OECD Guideline for Testing of Chemicals No. 301 F: "Ready Biodegradability: Manometric Respirometry Test", adopted July 17, 1992

**Purpose:** The test item Wacker BS 1701 was investigated for its ready biodegradability in a Manometric Respirometry Test over a period of 28 days. The biodegradation was followed by the oxygen uptake of the micro organisms during exposure. As a reference item Aniline was tested simultaneously under the same conditions as the test item, and functioned as a procedure control.

**Results:**

Biodegradation of Wacker BS 1701: After correction of the mean biochemical oxygen demand of the inoculum controls at the end of the 28-day exposure period degradation rates of 0 % and 2 % were found. The test item can therefore not considered to be ready biodegradable.

Biodegradation of Aniline: The reference item Aniline was sufficiently degraded to 81 % after 14 days, and to 104 % after 28 days of incubation, thus confirming the suitability of the used activated sludge inoculum.

Biodegradation in the Toxicity Control: In the toxicity control containing both, the test item and the reference item Aniline, 40 % biodegradation was noted within 14 days and 46 % biodegradation was determined after 28 days of incubation. Thus, the test item can be assumed to be not inhibitory on the activated sludge micro organisms.

## 2. Survey of the Study

### 2.1 General Information

| | |
|---|---|
| **Title:** | Ready Biodegradability of Wacker BS 1701 in a Manometric Respirometry Test |
| **Sponsor:** | Wacker-Chemie GmbH<br>Werk Burghausen<br>Johannes-Hess-Straße 24<br>84489 Burghausen<br>Germany |
| **Monitoring:** | Dr. Axel Bosch |
| **Test Item:** | Wacker BS 1701 |
| **Test Facility:** | Institut für Biologische Analytik und Consulting IBACON GmbH<br>Arheilger Weg 17<br>64380 Rossdorf<br>Germany |
| **IBACON-Project:** | 9545160 |

**Project Staff:**

| | |
|---|---|
| Test Facility Management: | Dr. Ralf Petto |
| Study Director: | Dr. Johannes Hertl |
| Technical Coordination: | Carina Weiss |
| Head of Quality Assurance Unit (QAU): | Dipl. Biol. Christiane Rutschmann-Fröhlich |
| Quality Assurance Unit Managers: | Dipl. Biol. Antje Pfützner<br>Dipl. Biol. Erika Schnellbächer |

**Schedule:**

| | |
|---|---|
| Study Initiation Date: | November 13, 2000 |
| Experimental Starting Date: | November 15, 2000 |
| Experimental Completion Date: | December 13, 2000 |
| Draft Report Date: | December 20, 2000 |
| Study Completion Date: | January 22, 2001 |

## 2.2 Good Laboratory Practice

This study was performed in compliance with:

- The OECD Principles of Good Laboratory Practice (as revised in 1997) and the
- Chemikaliengesetz (*'Chemicals Act'*) der Bundesrepublik Deutschland (ChemG), Anhang 1 (*'Annex 1'*), 1994/97.

This study was assessed in compliance with the study protocol and the IBACON Standard Operating Procedures. This study and/or test facility were periodically inspected by the Quality Assurance Unit (QAU) and the dates and the phases of the inspections are included in this final report. The data contained within this final report were audited in comparison to the raw data.

A quality assurance statement, signed by the Quality Assurance Unit, is included in this final report.

## 2.3 Archiving

The following data / sample(s) will be archived

for 15 years:
- all raw data
- the study protocol
- one certified copy of the final report

for at least 2 years:
- one sample of the test item and of the reference item

following the date on which the final report is audited by the Quality Assurance Unit at:

Institut für Biologische Analytik
und Consulting IBACON GmbH
Arheilger Weg 17
64380 Rossdorf
Germany

No raw data or material relating to the study will be discarded without the sponsor's prior consent.

## 2.4 Signatures

**Study Director:** Dr. Johannes Hertl

date: January 22, 2001

**Test Facility Management:** Dr. Ralf Petto

date: January 22, 2001

## 3. Quality Assurance Unit Statement

**Test Facility:**

Institut für Biologische Analytik
und Consulting IBACON GmbH
Arheilger Weg 17
64380 Rossdorf
Germany

IBACON Project: 9545160

Title of the Study: Ready Biodegradability of Wacker BS 1701 in a Manometric Respirometry Test

Test Item: Wacker BS 1701

Study Director: Dr. Johannes Hertl

**Study based Inspections**

| Phases inspected | Dates of QAU Inspections | Dates of Reports to Study Director and to Test Facility Management |
|---|---|---|
| Study Protocol | November 13, 2000 | November 13, 2000 |
| Experimental Phase | November 15, 2000 | November 15, 2000 |
| Draft Report | December 21, 2000 | December 21, 2000 |
| Final Report | January 22, 2001 | January 22, 2001 |

This statement confirms that the final report reflects the raw data.

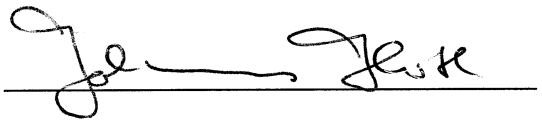
Dipl. Biol.
Christiane Rutschmann-Fröhlich

Quality Assurance Unit:

date: January 23, 2001

## 4. Statement of Compliance

| | |
|---|---|
| IBACON Project: | 9545160 |
| Title of the Study: | Ready Biodegradability of Wacker BS 1701 in a Manometric Respirometry Test |
| Test Item: | Wacker BS 1701 |
| Study Director: | Dr. Johannes Hertl |
| GLP-Regulations: | • The OECD Principles of Good Laboratory Practice (as revised in 1997) and the<br>• Chemikaliengesetz (*'Chemicals Act'*) der Bundesrepublik Deutschland (ChemG), Anhang 1 *('Annex 1')*, 1994/97. |
| Integrity of the Study: | This study performed in the test facility of IBACON, was conducted in compliance with the Good Laboratory Practice regulations. There were no circumstances that may have affected the quality or integrity of the study. |
| Study Director: | Dr. Johannes Hertl |



date: January 23, 2001

## 5. Objectives of the Study

**5.1 Title**

Ready Biodegradability of Wacker BS 1701 in a Manometric Respirometry Test

**5.2 Purpose**

The purpose of this study was to determine the ready biodegradability of the test item Wacker BS 1701. The test item was exposed to activated sludge from the aeration tank of a domestic waste water treatment plant for 28 days. The biodegradation was followed by the oxygen uptake of the micro organisms during exposure. As a reference item Aniline was tested simultaneously under the same conditions as the test item, and functioned as a procedure control.

This study is recognized by the OECD and EEC guidelines and should provide a rational basis to assess the ready biodegradation properties of the test item when incubated with activated sludge.

**5.3 Guidelines / Recommendations**

This study was designed to comply with the following methods:

- Commission Directive 92/69/EEC, Method C.4-D of July 31, 1992: Manometric Respirometry Test (EEC Publication No. L 383 A, December 1992).
- OECD Guideline for Testing of Chemicals No. 301 F: "Ready Biodegradability: Manometric Respirometry Test", adopted July 17, 1992.

## 6. Material and Methods

### 6.1 Test Item and Reference Item

**Test Item**

The test item and the information concerning the test item were provided by the sponsor:

| | |
|---|---|
| Name: | Wacker BS 1701 |
| Batch No.: | KH 02343 |
| Active Ingredient(s) / Purity: | alkylalkoxysilane / 98.53 %, (GC) |
| Certificate of Analysis / Date: | 20.07.2000 |
| Aggregate State at RT: | liquid |
| Molecular Weight: | 276.49 g/mol |
| Molecular Formula: | $C_{14}H_{32}O_3Si$ |
| Colour: | colourless |
| Density (at 25 °C): | 0.86 g/cm$^3$ |
| Solubility: | in water: insoluble |
| Stability: | pure: see expiry date<br>in water: not indicated by the sponsor |
| Expiry Date: | November 2001 |
| Storage: | in original container, at room temperature, in the dark |

**Reference Item**

The information concerning the reference item were provided by the manufacturer:

| | |
|---|---|
| Identity: | Aniline p.A. |
| Batch No.: | K25227461 |
| Expiry Date: | May 31, 2001 |
| Purity: | 99.5 %, freshly distilled August 16, 2000 |
| Certificate of Analysis Ref. Code / Date: | May 06, 1998 |
| Molecular Weight: | 93.13 g/mol |
| Storage: | in original container, at room temperature, in the dark |

**6.2 Test System**

| | |
|---|---|
| Species: | activated sludge, micro organisms from a domestic waste water treatment plant |
| Origin: | supplied by the sewage plant Groß-Zimmern, Germany |
| Conditioning: | The activated sludge used for this study was washed by centrifugation and the supernatant liquid phase was decanted. The solid material was resuspended in tap water and again centrifuged. This procedure was repeated twice. An aliquot of the final sludge suspension was weighed, dried and the ratio of wet sludge to its dry weight was determined. Based on this ratio, calculated aliquots of washed sludge suspension, corresponding to 1.5 g dry material per litre were mixed with test water (see 6.5) and then aerated until use. |

**6.3 Test Units**

| | |
|---|---|
| Type and Size: | Manometric Test System with test flasks containing a volume of 500 mL (see 6.8). |
| Identification: | Each test unit was uniquely identified with the study number, treatment and replicate number. |

**6.4 Test Conditions**

| | |
|---|---|
| Surrounding Type: | climatic chamber |
| Temperature: | 22 °C |
| Light Conditions: | darkness |
| pH-Value of Test Solutions: | 7.6 (measured at the start of the test) |

**6.5 Test Water**

Reconstituted Test Water: In deionized water analytical grade salts were added to prepare the following stock solutions:

a) 8.5 g $KH_2PO_4$, 21.75 g $K_2HPO_4$, 33.4 g $Na_2HPO_4$ x 2 $H_2O$, 0.5 g $NH_4Cl$ filled up with deionized water to 1000 mL volume

b) 22.5 g $MgSO_4$ x $7H_2O$ filled up with deionized water to 1000 mL volume

c) 36.4 g $CaCl_2$ x $2H_2O$ filled up with deionized water to 1000 mL volume

d) 0.25 g $FeCl_3$ x $6H_2O$ filled up with deionized water to 1000 mL volume

In order to avoid preparation of the stock solution d) immediately before use, one drop of concentrated HCl per litre was added.

10 mL of stock solution a) and 1 mL of the stock solutions b) - d) were combined and filled to a final volume of 1000 mL with deionized water.

### 6.6 Preparation of the Test Solutions

| | |
|---|---|
| Test Item (flasks 1 and 2): | 25.1 and 25.4 mg Wacker BS 1701, respectively and activated sludge at a concentration of 30 mg suspended solids per litre were filled up with test water to a volume of 244 mL. |
| Inoculum Control (flasks 3 and 4): | Activated sludge at a concentration of 30 mg suspended solids per litre was filled up with test water to a volume of 244 mL. |
| Procedure Control (flask 5): | 24.9 mg Aniline and activated sludge at a concentration of 30 mg suspended solids per litre were filled up with test water to a volume of 244 mL. The procedure control was also used for other projects which ran in parallel. |
| Abiotic Control (flask 6): | 25.2 mg Wacker BS 1701 filled up with test water (sterile filtered, 0.2 - 0.45 µm filter) to a volume of 244 mL. |
| Toxicity Control (flask 7): | 25.5 mg Wacker BS 1701, 24.8 mg Aniline and activated sludge at a concentration of 30 mg suspended solids per litre were filled up with test water to a volume of 244 mL. |

### 6.7 Course of the Test

| | |
|---|---|
| Preparation of Test Flasks: | The amounts of test item and reference item were directly weighed into the test flasks. The solutions were dispersed by stirring during the exposure period to achieve a homogeneous solution of the test item. |
| Incubation: | The closed test flasks were incubated in a climatic chamber under continuously stirring. The consumption of oxygen was determined by measuring the change of pressure in the flasks. Evolved carbon dioxide was absorbed in an aqueous solution (45 %) of potassium hydroxide. |
| Test Duration: | 28 days |

### 6.8 Test Parameters

| | |
|---|---|
| Measurement of Oxygen: | The change of pressure in the test flasks was measured by means of a manometric method (BSB/BOD-Sensor-System, Aqualytic, D-63263 Neu Isenburg, Germany) each day. |
| Temperature: | Temperature was measured each working day in the climatic chamber. |
| pH-Value: | pH-values were measured in all flasks at the start and end of the test using a pH-electrode ECM-Multi (Dr. Lange, D-40549 Düsseldorf, Germany). |

### 6.9 Result Evaluation

| | | |
|---|---|---|
| Definitions: | $ThOD_{NH4}$: | Theoretical Oxygen Demand<br>the total amount of oxygen required to oxy- |

dize a chemical completely. It is calculated from the molecular formula and expressed as mg oxygen required per mg test item.

BOD: Biochemical Oxygen Demand
the amount of oxygen consumed by micro organisms when metabolizing a test item; also expressed as mg oxygen uptake per mg test item.

10-day window: the 10 days immediately following the attainment of 10 % biodegradation

Calculation of BOD: The biodegradability (% BOD = mg $O_2$ per mg test item) exerted after each period was calculated as:

$$BOD = \frac{\text{mg } O_2 \text{ uptake of test item - mg } O_2 \text{ uptake of inoculum control}}{\text{mg test item in flask}}$$

The percentage biodegradation of the test item and of the reference item Aniline was calculated as:

$$\% \text{ degradation} = \frac{\text{BOD (mg } O_2\text{/mg test item or Aniline)}}{ThOD_{NH4} \text{ (mg } O_2\text{/mg test item or Aniline)}} \times 100\ \%$$

The $ThOD_{NH4}$ of Wacker BS 1701 was calculated to be 2.49 mg $O_2$/mg test item.

## 6.10 Validity Criteria of the Study

Inoculum Control: The oxygen demand of the inoculum control (medium and inoculum) was 25 mg $O_2$/L and thus not greater than 60 mg $O_2$/L within 28 days.

pH-Value: The pH-value of the test item flasks at the end of the test was pH 7.4 and is thus within the range of pH 6.0 - 8.5.

Reference Item: The percentage degradation of the reference item Aniline reached the level for ready biodegradability (about 60 %) within 5 days.

## 6.11 Deviations to the Study Protocol

There were no deviations to the study protocol.

## 7. Results and Discussion

### 7.1 Biodegradation of Test Item

| | |
|---|---|
| Percentage Biodegradation: | Under the test conditions the percentage biodegradation of Wacker BS 1701 reached 0 % and 2 % after 28 days of exposure. The results are represented in Tables 1 and 2 and Figure 1. |
| Conclusion: | The test item can not considered to be ready biodegradable. |

### 7.2 Biodegradation of Reference Item Aniline

| | |
|---|---|
| Percentage Biodegradation: | The reference item Aniline was sufficiently degraded to 81 % after 14 days, and to 104 % after 28 days of incubation. The results are represented in Tables 1 and 2 and Figure 1. |
| Conclusion: | The percentage biodegradation of the reference item confirms the suitability of the used activated sludge inoculum. |

### 7.3 Biodegradation in the Toxicity Control

| | |
|---|---|
| Percentage Biodegradation: | In the toxicity control containing both, the test item and the reference item Aniline, 40 % biodegradation was noted within 14 days and 46 % biodegradation was determined after 28 days of incubation. The results are represented in Tables 1 and 2. |
| Conclusion: | According to the test guidelines the test item can be assumed to be not inhibitory on the activated sludge micro organisms because degradation was > 25 % within 14 days. |

### 7.4 Abiotic Control

| | |
|---|---|
| Oxygen Demand: | The oxygen demand in the abiotic control was below the oxygen demand in the control flasks (see Table 1). |

## 8. References

1. Chemikaliengesetz der Bundesrepublik Deutschland (ChemG), Anhang 1, in der Fassung der Bekanntmachung vom 25. Juli 1994 (BGBl. I S. 1703) mit Änderungen vom 27. September 1994 (BGBl. I S. 2705) und 14. Mai 1997 (BGBl I S. 1060)

2. Commission Directive 92/69/EEC, Method C.4-D of July 31, 1992: Manometric Respirometry Test (EEC Publication No. L 383 A, December 1992)

3. OECD Guideline for Testing of Chemicals No. 301 F: "Ready Biodegradability: Manometric Respirometry Test", adopted July 17, 1992

4. The OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997 [C(97)186/Final], Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998

5. Simple determination of biodegradability in accordance with OECD 301 F. Melliand reprint 10/1996, E 148-E 150

## 9. Distribution of the Final Report

| | |
|---|---|
| Sponsor: | the original final report |
| IBACON: | one certified copy of the final report |

# Appendix

**Table 1.** Cumulative biochemical oxygen demand (mg $O_2$/L) in test flasks during the test period of 28 days

| Time | Flask No. | | | | | | |
|---|---|---|---|---|---|---|---|
| (days) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 5 | 0 | 3 | 0 | 0 | 0 | 0 |
| 3 | 5 | 0 | 4 | 3 | 105 | 0 | 35 |
| 4 | 5 | 0 | 6 | 3 | 140 | 0 | 135 |
| 5 | 5 | 5 | 6 | 4 | 155 | 0 | 150 |
| 6 | 5 | 5 | 4 | 4 | 165 | 0 | 165 |
| 7 | 5 | 5 | 9 | 9 | 180 | 0 | 170 |
| 8 | 10 | 5 | 6 | 9 | 185 | 0 | 185 |
| 9 | 10 | 10 | 12 | 12 | 190 | 0 | 190 |
| 10 | 10 | 10 | 9 | 9 | 195 | 0 | 190 |
| 11 | 15 | 10 | 10 | 12 | 200 | 0 | 200 |
| 12 | 15 | 10 | 12 | 13 | 205 | 0 | 205 |
| 13 | 15 | 15 | 12 | 13 | 210 | 0 | 210 |
| 14 | 15 | 15 | 13 | 16 | 215 | 0 | 215 |
| 15 | 15 | 15 | 13 | 16 | 220 | 0 | 220 |
| 16 | 20 | 15 | 16 | 18 | 225 | 0 | 225 |
| 17 | 20 | 15 | 18 | 18 | 235 | 0 | 230 |
| 18 | 20 | 20 | 19 | 19 | 240 | 2 | 230 |
| 19 | 20 | 20 | 19 | 19 | 245 | 3 | 235 |
| 20 | 20 | 20 | 18 | 19 | 250 | 3 | 240 |
| 21 | 20 | 20 | 21 | 21 | 255 | 6 | 245 |
| 22 | 25 | 20 | 19 | 21 | 260 | 7 | 250 |
| 23 | 25 | 20 | 21 | 21 | 265 | 7 | 250 |
| 24 | 25 | 20 | 22 | 22 | 275 | 9 | 255 |
| 25 | 25 | 20 | 21 | 22 | 280 | 10 | 255 |
| 26 | 25 | 25 | 24 | 24 | 280 | 12 | 255 |
| 27 | 25 | 25 | 24 | 25 | 280 | 13 | 255 |
| 28 | 30 | 25 | 25 | 25 | 280 | 15 | 255 |

flasks 1 and 2: Test item
flasks 3 and 4: Inoculum control
flask 5: Aniline (Procedure control)
flask 6: Abiotic control
flask 7: Toxicity control

**Table 2.** Percentage biodegradation ($BOD/ThOD_{NH4}$) of test item, of Aniline and of the toxicity control

| Time | Percentage BOD | | | |
|---|---|---|---|---|
| (days) | Wacker BS 1701 | | Aniline | Toxicity control |
| | flask 1 | flask 2 | flask 5 | flask 7 |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 1 | -1 | -1 | 0 |
| 3 | 1 | -1 | 41 | 6 |
| 4 | 0 | -2 | 55 | 26 |
| 5 | 0 | 0 | 61 | 29 |
| 6 | 0 | 0 | 65 | 32 |
| 7 | -2 | -2 | 70 | 32 |
| 8 | 1 | -1 | 72 | 35 |
| 9 | -1 | -1 | 72 | 35 |
| 10 | 1 | 1 | 76 | 36 |
| 11 | 1 | -1 | 76 | 37 |
| 12 | 1 | -1 | 78 | 38 |
| 13 | 1 | 1 | 80 | 39 |
| 14 | 0 | 0 | 81 | 40 |
| 15 | 0 | 0 | 83 | 41 |
| 16 | 1 | -1 | 85 | 41 |
| 17 | 1 | -1 | 88 | 42 |
| 18 | 0 | 0 | 90 | 42 |
| 19 | 0 | 0 | 92 | 43 |
| 20 | 1 | 1 | 94 | 44 |
| 21 | 0 | 0 | 95 | 44 |
| 22 | 2 | 0 | 98 | 46 |
| 23 | 2 | 0 | 99 | 45 |
| 24 | 1 | -1 | 103 | 46 |
| 25 | 1 | -1 | 105 | 46 |
| 26 | 1 | 1 | 104 | 46 |
| 27 | 0 | 0 | 104 | 46 |
| 28 | 2 | 0 | 104 | 46 |

$ThOD_{NH4}$ of Wacker BS 1701: 2.49 mg $O_2$/mg test item

**Table 3.** pH-values at the end of the test

| flask No. | Treatment | pH-value |
|---|---|---|
| 1 | Test item | 7.4 |
| 2 | Test item | 7.4 |
| 3 | Inoculum control | 7.5 |
| 4 | Inoculum control | 7.5 |
| 5 | Procedure control (Aniline) | 7.0 |
| 6 | Abiotic control | 7.6 |
| 7 | Toxicity control | 7.0 |

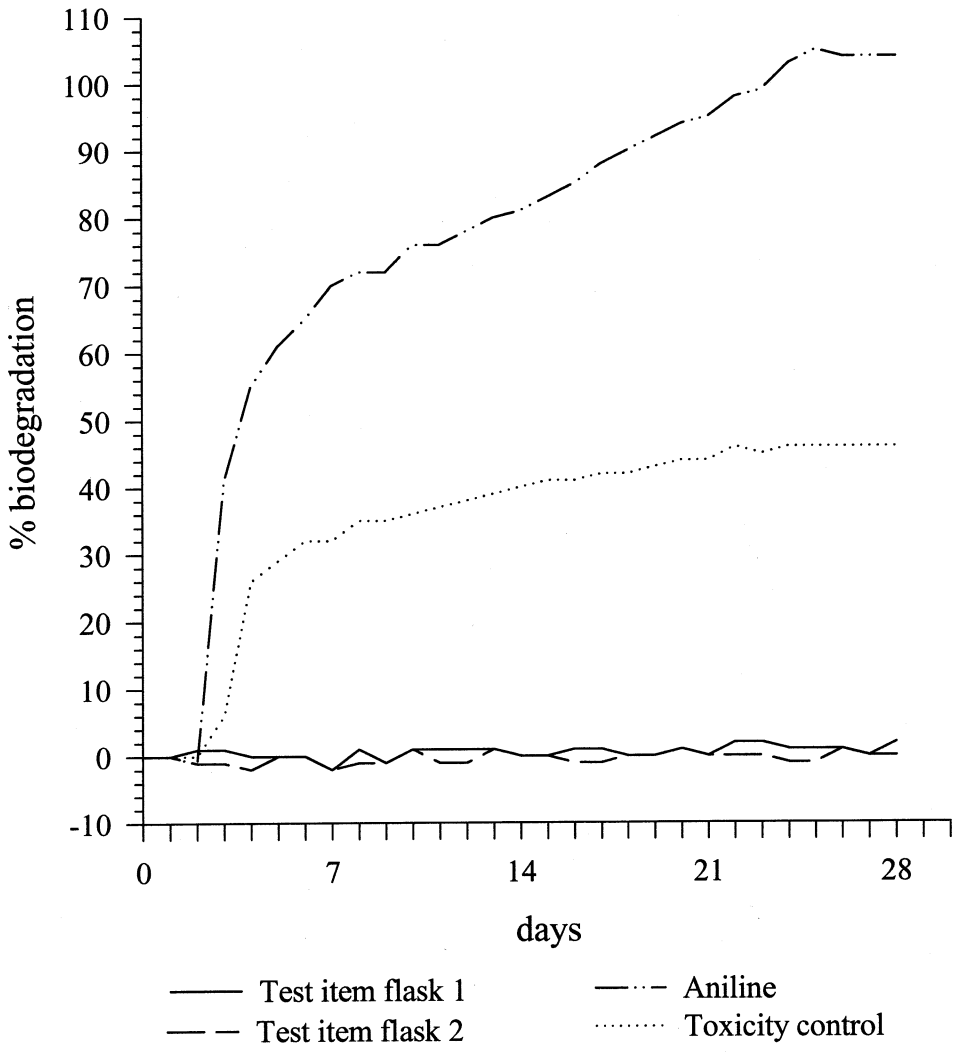



**Figure 1.** Biodegradation of Wacker BS 1701, of the reference item Aniline and of the toxicity control during the exposure period of 28 days